

Temperature induced aggregation in aqueous solution of a series of PEO–PPO–PEO copolymers

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Abstract

The temperature induced aggregation phenomena of a series of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers (poloxamers) were investigated using dye incorporation UV spectroscopy, over a range of mass concentrations ($0.1\text{--}15\text{ mg ml}^{-1}$). Initial experiments were performed using two different dyes (1,6-diphenyl-1,3,5-hexatriene (DPH) and I_2) so that the performance of each indicator for studying poloxamer aggregation could be determined. Subsequently, the onset temperatures (T_o) and temperature maxima (T_m) for the aggregation processes of 19 poloxamers were determined, using I_2 as an indicator. The aggregation temperature was observed to be dependent on both molecular mass and solution concentration for polymers comprising the same percent PEO, the aggregation temperature decreasing with increasing mass and/or increasing concentration. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Temperature induced aggregation; Dye incorporation UV spectroscopy; Onset temperature; Temperature maxima; Copolymers

1. Introduction

Block copolymers comprising of polyethylene oxide (PEO) and polypropylene oxide (PPO), in the form $(\text{PEO})_A\text{--}(\text{PPO})_B\text{--}(\text{PEO})_A$, where A and B represent the number of monomer units in each polymeric block, are commercially available, and

represent the earliest example of commercially produced block copolymers, first being manufactured in 1950 (Schmolka, 1977). They are termed Pluronic (BASF, Wyandotte, N.J.), Synperonic PE (ICI, Cleveland, UK) or poloxamers, and have found numerous industrial applications, mainly because of the varying hydrophilic/hydrophobic balance, and range of molecular weights, obtainable by alteration of the PEO/PPO ratio.

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Typically, they are employed pharmaceutically as stabilisers, emulsifiers or carriers in cosmetic and therapeutic treatments (Florence and Attwood, 1988; Schmolka, 1991). They have also been used as microcarriers for the systemic administration of novel therapeutic agents (Johnston and Miller, 1985), an application made possible by the aggregates these systems form at higher solution temperatures or concentrations.

There are two types of nomenclature used in the literature for the poloxamers;

(i) Paab where P = Pluronic or poloxamer; $aa \times 100 =$ Molecular mass of PPO moiety; $b \times 10 =$ Percent by mass of PEO (a *b*-series member).

(ii) Xab or Xaab where X = F (Flake), L (Liquid) or P (Paste); a or $aa \times 5 =$ Approximate number of PPO units; $b \times 10 =$ Percent by mass of PEO (a *b*-series member).

Nomenclature (ii) is used for naming industrially produced polymers. So, for example, P237 (i) or F-87 (ii) would be a solid product with approximately 40 PPO units, having a PPO moiety of molecular mass 2300 and consisting of 70% PEO (a member of the 7-series). Nomenclature (ii) will be used throughout this paper.

The poloxamers undergo many transitions in aqueous solution, mainly because of the hydrophobicity of the central PPO block, and have been the subject of much discussion within the literature. In particular, it has been observed that the poloxamers will aggregate (commonly referred to as micellisation) with an increase in solution temperature or concentration (Reddy et al., 1990; Brown et al., 1991; Linse and Malmsten, 1992; Beezer et al., 1994; Alexandridis and Hatton, 1995; Chu, 1995). These aggregates arise as a result of dehydration of the PPO moieties, and are assumed to consist of a PPO core surrounded by a corona of PEO, and have been well documented (Chu, 1995).

Since different poloxamers generally vary widely in their physical properties, it is usually not feasible to interchange one poloxamer for another in a particular application and, more often than not, trends in a particular property are observed across a series of poloxamers of related composition (Schmolka, 1977, 1991). A complete understanding of the relationships between structure

and physical properties would enable specific poloxamers to be designed for specific applications.

Many techniques have been used to study poloxamer aggregation, but few papers present data representing a systematic study of the aggregation phenomena of a large number of poloxamers. The most complete data set published was recorded using HSDSC (Beezer et al., 1994) and represents the aggregation temperatures for twenty seven poloxamers, at only one concentration (5 mg ml^{-1}). Since the poloxamers are widely used in pharmaceutical products, there is a need for a study that incorporates a large number of polymers, investigated over a range of concentrations, so that comparisons may be made between polymers that are structurally different.

In this work, the aqueous phase aggregation of nineteen poloxamers will be studied, using temperature scanning diode-array UV spectroscopy. Diode-array spectroscopy offers considerable sensitivity, while being cheap and easy to run. Since the poloxamers have no UV chromophores, we enhanced the resolution of the technique (over simple scattering) via the use of an indicator.

Many indicators may be used to study poloxamer aggregation and, for this work, two indicators, I_2 , and 1,6-diphenyl-1,3,5-hexatriene (DPH) will be employed. We have shown previously how I_2 can be employed to study poloxamer aggregation (Gaisford et al., 1995, 1997), while the use of DPH to study poloxamer aggregation has been reported recently by Alexandridis (Alexandridis and Hatton, 1995; Alexandridis et al., 1995a,b). Aggregation temperatures will be determined for nineteen members of the poloxamer series, at mass concentrations ranging from $0.1\text{--}15 \text{ mg ml}^{-1}$.

2. Materials and methods

2.1. Materials

The poloxamer samples (250 g) were donated by ICI (Cleveland, UK) and were used as received. The nineteen poloxamers selected gave a representative sample set, containing examples of

every series of the commercially available polymers. The poloxamer samples were stored at room temperature and were kept away from light sources.

The components of the indicator solutions, iodine (32614-3), potassium iodide (22194-5) and 1,6-diphenyl-1,3,5-hexatriene (DPH) (20800-0) were purchased from Aldrich, and were used as received. Aqueous solutions were made up in deionised water, obtained by passing distilled water through an Elgastat Micromeg ion exchange column. DPH solutions were prepared in ALR grade methanol. Data were recorded using the dedicated Hewlett–Packard software, and data analysis was performed using the mathematical software package ORIGIN™ (Microcal Software, MA).

Poloxamer solutions were prepared by dissolution of a known weight of polymer into doubly distilled water (10 ml) at 6°C. The low solution temperature aided the dissolution process. All poloxamer solutions were used within 24 h of preparation, and were rotated end over end three times before use to ensure complete dissolution, without creating a foam. Solutions were prepared at mass concentrations between 0.1–15 mg ml⁻¹.

The KI/I₂ standard was prepared by dissolution of iodine (1 g) and potassium iodide (2 g) in doubly distilled water (100 ml) at 25°C, and was kept away from light sources. A stock solution of 0.4 mM DPH standard was prepared in methanol, and was kept away from light sources.

Dependent on the poloxamer concentration, between 10–50 µl of the iodine standard was added to 10 ml of the poloxamer solution, the solution being rotated end over end three times to ensure complete mixing. For the DPH experiments, between 100–200 µl of the DPH/methanol standard was added to 10 ml of the poloxamer solution, resulting in a copolymer solution containing 1–2% v/v methanol and 0.004–0.008 mM DPH.

The absorbance at 366 nm (iodine), 356 nm (DPH), of the resulting solution was recorded over a certain temperature range, dependent on the poloxamer being studied, at a scan rate of 0.33°C min⁻¹ using the spectrometer. The temperature limits of the spectrometer cell were 10–95°C, and the temperature range chosen for a

particular polymer was based on the value obtained for the transition observed for that poloxamer via HSDSC (Beezer et al., 1994). A sealed quartz cuvette was employed to minimize solvent and iodine loss, and the solutions were stirred at 200 rpm, using a 5 mm magnetic stirring bar, to ensure there were no temperature gradients within the sample.

2.2. Methods

2.2.1. Iodine

This indicator solution was first proposed by Baleux (1972), and was chosen for its ease of use. We have detailed previously how this indicator may be used to study poloxamer aggregation (Gaisford et al., 1995, 1997), by following the change in absorption intensity with temperature as iodine is incorporated into the hydrophobic core of the poloxamer aggregate. From the resulting trace, it is possible to determine two parameters; the onset temperature of aggregation, T_o , and the temperature of maximum aggregation, T_m . T_m values represent the temperature at which the rate of aggregation is at a maximum and may be compared with literature HSDSC data. The determined T_m values are consistent with those determined previously for ICI products by HSDSC (Beezer et al., 1994). Some published data suggest that iodine interacts with PPO in aqueous solution (Binana-Limbelé et al., 1991) but we believe that our observations are good evidence that, under these conditions, iodine has no observable physical affect upon the aggregation process.

2.2.2. DPH

Recent work has shown that the fluorescent dye DPH may be similarly employed to study poloxamer aggregation (Alexandridis et al., 1994; Alexandridis and Hatton, 1995; Alexandridis et al., 1995a,b). DPH works via a similar principle to the iodine indicator solution. When the dye is solubilised in a hydrophobic environment, the absorption maximum changes from that observed in aqueous solution. Aggregation can therefore be studied by following the increase in absorption intensity with increasing solution temperature.

Unlike iodine, DPH is insoluble in water, and must be added as a methanolic solution. The authors of the above work state that, at such low added concentrations (1% v/v), the added methanol does not affect the aggregation process. Typical plots of absorbance versus temperature, for 5 mg ml⁻¹ F-87 with DPH, are shown in Fig. 1, the experiment being conducted with two concentrations of added indicator. As for the iodine experiments, onset temperatures (T_o) of aggregation and maximum temperatures (T_m) of aggregation can be determined.

In contrast to the results obtained for the iodine indicator, there is a marked effect of DPH concentration on the form of trace obtained, Fig. 1, although there is also a difference in the methanol concentration in the two cases. The T_o values obtained from each experiment are moderately close, (40.2°C–0.004 mM DPH, 41.9°C–0.008 mM DPH), suggesting that the onset of poloxamer aggregation is slightly affected by the presence of DPH, while the T_m values are markedly different (42.5°C–0.004 mM DPH, 45.7°C–0.008 mM DPH). In publications on DPH solubilisation, Alexandridis suggests that the second inflection in the temperature-absorbance trace arises from complete DPH uptake (Alexandridis et al., 1994). An increase in DPH concentration should therefore increase the temperature at which the second inflection arises, so this hypothesis appears valid, and would lead to the difference in the observed T_m values.

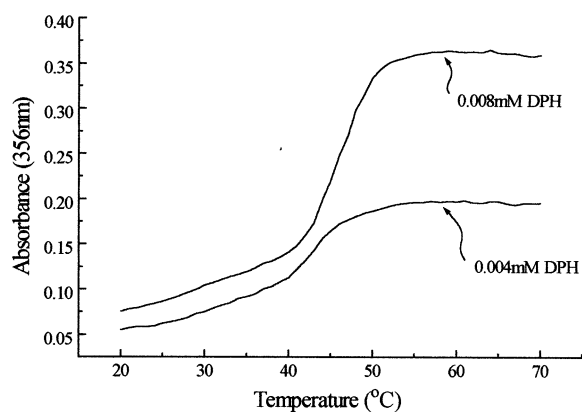


Fig. 1. Absorbance versus temperature for 5 mg ml⁻¹ F-87 using DPH, at two concentrations, as an indicator.

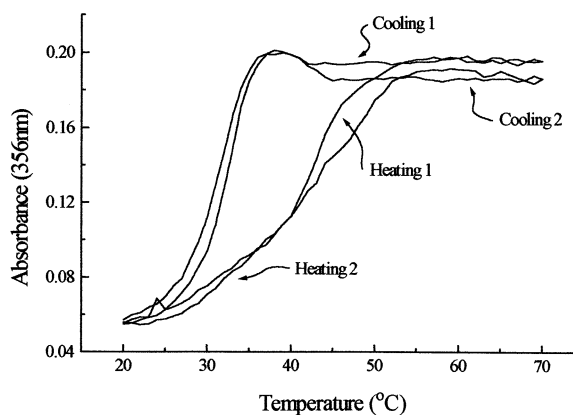


Fig. 2. Absorbance versus temperature for repeated heating/cooling scans of 5 mg ml⁻¹ F-87 using DPH as an indicator.

Repeat heating/cooling scans using DPH as an indicator for 5 mg ml⁻¹ F-87 reveal that DPH does not give traces which are thermally reversible, Fig. 2. The cooling traces suggest that the aggregates dissociate over a narrower temperature range than that over which they were formed. This implies that, once initiated, the rate of aggregate breakdown is faster than the rate of aggregate formation. Since, as has been shown previously, DSC experiments provide evidence that poloxamer aggregation is thermally reversible, in the absence of any added indicator (Armstrong et al., 1994), it can be concluded that the presence of DPH affects the aggregation process, presumably because of the presence of mixed aggregates.

It should be noted that the T_o value obtained for F-87 using DPH is markedly higher than that obtained using iodine (cf. 40.2°C–DPH, 35.3°C–iodine, Table 1). This difference is also reflected in the HSDSC data (Beezer et al., 1994), suggesting that iodine can be solubilised by smaller aggregates and, as noted above, appears not to influence the aggregation process.

3. Results

Onset (T_o) and maximum (T_m) temperatures of aggregation determined for the poloxamer samples are given in Table 1. In general, higher

Table 1
 T_o/T_m values ($\pm 0.5^\circ\text{C}$) for 19 members of the poloxamer series

Poloxamer	Poloxamer solution concentration (mg ml^{-1})						
	0.1	0.5	1	2	5	10	15
L-121	←			No Transition			→
L-42	←			No Transition			→
L-62	50.0/58.2	39.4/ND	37.5/40.3 ^b	35.5/35.7 ^b	31.3/38.3	28.5/32.7	28.0/28.6
L-92	32.8/37.1	27.8/32.3	27.0/31.9	25.4/30.3	24.4/29.2	20.9/27.4	19.6/25.5
P-103	22.9/27.5	21.9/25.3	21.8/26.3	20.6/25.3	20.4/22.9	18.7/20.8	18.1/19.9
L-44	77.1/ND	62.7/ND	59.4/68.1	54.0/64.8	45.7/52.6	42.9/49.3	40.9/46.7
L-64	49.7/57.1	42.1/47.2	40.5/47.9 ^c	36.1/41.4 ^c	33.3/37.8	31.2/35.4	30.1/33.1
P-84	32.0/35.8	28.3/31.8	28.0/31.7	26.4/29.7	24.7/27.2	23.1/25.4	21.2/24.5
P-94	29.6/33.7	26.9/30.3	26.4/30.3	25.3/28.5	24.3/26.8	22.5/24.7	19.9/23.6
L-35	No Peaks	74.9/ND	79.3/ND	67.6/77.7	55.1/62.5	52.6/57.5	ND/54.7
P-85	30.5/ND ^a	29.4/34.8	26.9/32.1	25.6/30.2	24.7/28.8	22.0/25.0	20.6/24.3
P-105	23.5/ND ^a	23.8/27.5	22.4/26.3	21.9/26.2	17.1/22.6	15.0/18.5	14.3/17.6
F-77	53.6/ND ^a	47.8/54.8	44.9/52.3	41.7/48.8	38.7/45.0	33.3/42.0	32.9/40.2
F-87	49.3/ND ^a	43.2/48.7	41.4/46.7	38.3/43.3	35.3/41.1	32.1/38.4	29.2/36.7
F-127	No Peaks	24.0/27.3	22.4/27.0	22.3/26.6	22.2/25.7	21.2/24.1	20.5/22.8
F-38	No Peaks	No Peaks	No Peaks	71.3/79.3	66.0/72.3	59.7/67.3	58.7/64.8
F-68	No Peaks	65.3/70.9	62.8/68.7	58.3/64.1	54.2/58.6	49.4/55.5	49.2/53.5
F88	35.1/ND ^a	32.6/36.4	30.7/35.9	30.0/34.6	29.7/33.7	27.6/31.6	26.4/29.2
F-108	No Peaks	27.1/31.2	25.9/30.2	24.6/29.2	22.8/28.5	21.4/25.3	18.5/22.1

^a These polymers gave very shallow transitions at low concentrations from which T_m values could not be determined.

^{b/c} These polymers gave rise to pre- and main transitions (see main text).

resolution traces were obtained at higher solution concentrations, some traces for polymers obtained at concentrations of 0.1 mg ml^{-1} proving too shallow to analyse. Low molecular weight 1 and 2-series poloxamers showed no observable transitions over the study temperature, or concentration, range. Polymers with relatively low transition temperatures were generally observed to precipitate (cloud out) at higher ($> 60^\circ\text{C}$) solution temperatures and, since both L-121 and L-42 were observed to cloud out in each experiment, it can be concluded that these polymers aggregate at temperatures less than 10°C .

Both T_o and T_m values were observed to decrease with increasing polymeric mass, or increasing polymeric concentration, within any one particular series. For most of the study polymers, it was observed that plots of transition temperature versus polymer concentration approached zero concentration asymptotically. It appears experimentally that iodine incorporation spectroscopy is not sensitive enough to allow the

study of polymer solutions of concentrations less than 0.1 mg ml^{-1} , so a more detailed investigation of this region was not possible. The data suggest, however, that at low concentrations, ($< 2 \text{ mg ml}^{-1}$), the poloxamers exhibit behaviour analogous to that shown by weak electrolytes. As the concentration of the polymer solution approaches infinite dilution, the individual polymer molecules become separated to such an extent that they can no longer interact. Since it is presumed that aggregation requires intermolecular interactions, it is theoretically impossible to extrapolate a plot of transition temperature versus concentration to infinite dilution.

Knowledge of how transition temperatures vary with poloxamer structure should allow a structure-activity relationship (SAR) to be determined, allowing transition temperatures to be estimated for any poloxamer of known structure. Neither the variation of transition temperature values with PEO mass content for poloxamers comprising identical PPO moieties, nor the variation of tran-

sition temperature values with PPO mass content for poloxamers comprising identical PEO moieties show any form of systematic relationship. However, within any one particular series, the transition temperatures determined at a fixed concentration decrease (approximately) linearly with an increase in molecular mass. Since it is possible to construct such a plot for most poloxamer series, from the data presented here, knowledge of the structure of a particular poloxamer should allow an estimate to be made of its transition temperature.

It is interesting to note that two of the study poloxamers exhibited anomalous behaviour, both of these polymers giving traces that showed characteristics of double transitions. For low concentration solutions of L-62, a small pre-transition was observed before the onset of aggregation, this pre-transition becoming more prominent at lower solution concentrations. Upon cooling a particular L-62 solution to room temperature, and repeating the experiment, the pre-transition remained present, and increased slightly in magnitude. After several heating/cooling cycles, no further change was observed in the pre-transition, Fig. 3. It is possible that the pre-transition arises as a result of the presence of a small amount of impurity in the L-62 sample, the impurity having a higher molecular weight than L-62, since it appears to aggregate at a lower temperature. At higher polymer concentrations, the main aggrega-

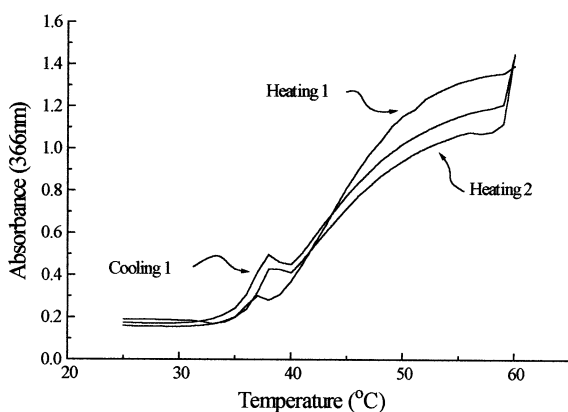


Fig. 3. Absorbance versus temperature for repeated heating scans of 2 mg ml^{-1} L-62.

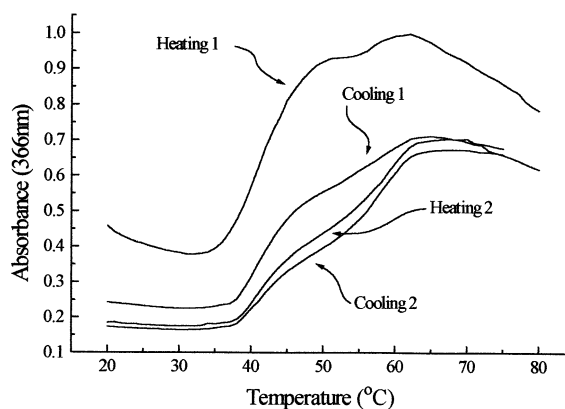


Fig. 4. Absorbance versus temperature for repeated heating scans of 2 mg ml^{-1} L-64.

tion transition becomes broader, and the pre-transition is no longer observed. The pre-transition was also observed for binary mixtures containing L-62.

Solutions of L-64 also showed the presence of a pre-transition but, in this case, the pre-transition formed a shoulder on the main transition, Fig. 4. The pre-transition remained observable after repeated heating/cooling cycles for any particular L-64 sample. Such anomalous behaviour was noted for aqueous solutions of L-64 by Zhou (Zhou and Chu, 1988) and it was suggested that this behaviour was caused by the presence of some insoluble impurity.

3.1. Aggregation thermodynamics

The main approaches to the thermodynamic analysis of polymeric aggregation have been summarised previously (Alexandridis and Hatton, 1995), whereby a plot of $\ln(X)$ versus $1/T_o$, where X is the total polymer concentration expressed in mole fraction units, should yield a straight line, the value of ΔH° being determined from the gradient.

This type of analysis (van't Hoff) has been widely employed to interpret poloxamer aggregation data derived via a number of different techniques. It is a requirement of the van't Hoff analysis that the process under study proceeds via a mechanism of the type $A \rightarrow B$, i.e. that the

mechanism is a simple, two-state process. Evidence suggests that poloxamer aggregation is a multi-step process (Wong, 1974; McDonald and Wong, 1977). At low solution temperatures (less than T_o) each poloxamer molecule is present in an equilibrium solvated state and, at low mass concentrations, there are no intermolecular interactions. As the solution temperature is increased, PEO chain entanglement and PPO dehydration lead to the formation of small aggregates. At higher solution temperatures the average size of the aggregates increases until precipitation occurs, resulting in both the cloud point and gel phenomena that are associated with aqueous poloxamer solutions.

The increase in average aggregate size, at higher solution temperatures, is observed to be both calorimetrically and spectrometrically 'silent' since, in both cases, it is PPO dehydration, and subsequent formation of hydrophobic regions, that gives rise to a detectable change in a physical property. The observation that phase transitions observed via HSDSC and UV spectroscopy for both heating and cooling scans are mirror images provides good evidence that poloxamer aggregation proceeds via a multi-step process. If aggregation occurred in a single step process, the onset temperatures of both the forward and reverse transitions would, ideally, be the same (cf. In an ideal system, the onset of water melting would be 0°C , the onset of water freezing would be 0°C . The two traces obtained, although involving the same enthalpy change, of opposite sign, would not be mirror images, but would be displaced on the temperature axis). The reversibility can be explained by imagining aggregation proceeding via a series of discreet steps. At equilibrium, the forward and reverse onset temperatures for each step would be the same but, since each step would encompass a very small temperature range, the observed trace would be composed of many such equilibria, and appear reversible.

Moreover, it is known that the organisational states of poloxamers in solution, over a range of mass concentrations, differ significantly; the initial state of the polymer solution changing from a homogeneous dilute solution to a gel as the concentration is increased. Thus, in the van't Hoff

analysis, state A varies as a function of concentration as does, necessarily, state B as a function of temperature. It follows that the processes under study at each poloxamer concentration are different, and analysis of such data using a van't Hoff analysis is inappropriate. The enthalpy changes associated with aggregation at different poloxamer concentrations are likely to be similar, because the phenomena observed have similar molecular interaction origins, and, it is possible that, neither HSDSC nor UV spectroscopy are sensitive enough to detect these changes. This insensitivity to fine structure contributes to the apparent correspondence of these data with the van't Hoff isochore. Since ΔG° must be negative, because aggregation is spontaneous, and the enthalpy values are always positive, it can be concluded that poloxamer aggregation is entropy driven, a conclusion that has been drawn previously (Alexandridis and Hatton, 1995).

4. Summary

Iodine incorporation UV-spectroscopy has proved to be an excellent tool to study the aggregation phenomena of a series of poloxamers. The technique is cheap, simple, reliable and reproducible. An alternative indicator to iodine was studied but, overall, it appeared that iodine was the most suitable candidate, giving both high resolution spectra while not appearing to affect significantly the aggregation process. The processes by which poloxamers aggregate have been reported widely, and the overall mechanism is generally accepted. It was not the purpose of this study to investigate mechanistically the aggregation process, rather to determine some of the temperature dependent properties of individual members to form the basis of an SAR.

The study of 19 members of the poloxamer series has resulted in a large number of data, and the temperature dependent behaviour of each polymer, over a wide concentration range, being determined. The organisational state of each of the study polymers at given temperature or concentration is now known, and this may be of use when considering the application of a poloxamer

in a particular formulation or product. Moreover, it would seem reasonable that the properties of a previously unstudied poloxamer could be predicted, as long as the percent PEO content and total molecular weight are known. Evidence suggests that aggregation is not a single-step process, but proceeds via a series of steps. If this is the case, then a thermodynamic van't Hoff analysis, widely used by other workers, is inappropriate.

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